APPARENT TISSUE MODULUS FOR MICRO/NANO PILLAR ARRAYS

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ABSTRACT

Polymeric culture platforms with micro/nano topographies have provided a powerful approach to modulate cell to substrate interactions. Substrate stiffness and topography has been widely explored in order to study physical cues that cell exert. Cells respond to chemical and mechanical signals from surrounding microenvironments. While chemical signals have been well studied in the last decade [1,2], mechanical cues are also very important to cell growth owing to a surface rigidity and compatibility. It has been recognized that substrate stiffness has a significant effect on traction forces as well as cell differentiation, growth, and mobility[3]. The goal of this given study is to investigate beam deformation as stiffness element in order to simulate apparent elastic modulus that focal adhesion regions would signal during cell culturing. Furthermore, this approximation would be a superior tool that helps cell mechanosensing investigators to design efficient substrates that match the tissue's elastic moduli.

Keywords: micro/nano Pillar deflections, Tissue elastic modulus, Timoshenko Beam.

INTRODUCTION

Cell to substrate interactions have got attention recently in both academic laboratories and industry. Cells that make tissues are adherent to their surrounding extracellular matrix (ECM). ECM is made up of non-cellular components present with all tissues and organs that provide both physical support and mechanosensing distribution in both directions [4]. Cells explore cultured substrate via traction forces in order to migrate across their environment, and also mechanically sense the characteristics of the substrate.

Substrate stiffness plays an important role in cells mechanosensing to determine a proper microenvironment during the cell cluttering process. It has been recognized that substrate stiffness has a significant effect on traction forces as well as cell differentiation[3]. Topography on substrates could be used to sense cellular forces on cells by modeling each pillar as a cantilever beam. Considerably the shape cross section of the pillar is mainly a high factor that could control substrate rigidity. Fabrication of substrates with micro and nano pillars have made it possible to estimate cell traction forces through pillar deflection [2].Therefore, it requires the development of novel mold fabrication with micro/nanostructured features to fulfill the demand of petri-dish industry.

Micro/Nano Pillars Bending Mechanism

- **Euler – Bernoulli Approximation**

Bending mechanism can be established from Euler-Bernoulli (E-B) beam bending theory, which can be used to determine the amount of deflection of a beam when a load is applied. The deflection behavior is a function of pillars geometry and material. Consider a slender beam in Figure 1 with length, *L*, and diameter, *D*, with a concentrated load *F*, acting at the free end, such that it deflects by δ in the *y* direction as shown in Figure 1. The pillar bending due to the force can be approximated using the Euler-Bernoulli model which assumes:

- An elastic beam where the deflections are small.
- Rigid cross sections that do not deform under to the transverse (or axial) load.
- Cross sections that remain planar and normal to the neutral axis of the beam

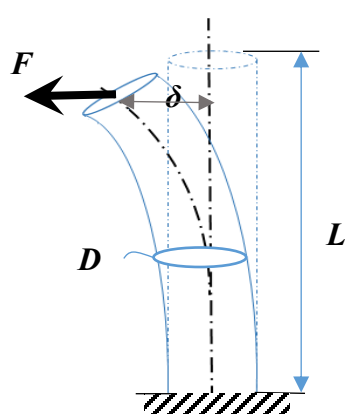

Figure 1: Schematic of Cantilver Beam deflection

The cantilever deflection can be derived from Euler Bernoulli equation based on the applied load at the tip and cross section of the beam as follows:

$$\boldsymbol{\delta} = \frac{F\,L^3}{3EI} \qquad (1)$$

Where F is applied load, L is the height of the pillars, E is the elastic modulus of pillars, I is the area moment of inertia attributed to pillars cross-section.

- **Timoshenko Beam Approximation**

In cases when the beam does not satisfy the slenderness criterion (L/D < 10), which is the typical case for micro-fabricated pillars, shear deformation may be significant. This can be accounted for by including the Timoshenko beam approximation term[5,6] :

$$\boldsymbol{\delta} = \boldsymbol{F}\frac{L^3}{3EI} + \boldsymbol{F}\frac{\alpha L}{GA} \qquad (2)$$

Where the new terms, $\boldsymbol{\alpha}$ is the shear coefficient specific to a cross section, *G* is the shear modulus, and *A* is the cross sectional area.

For a cylindrical pillar (beam) the area moment of inertia is

$$\boldsymbol{I} = \frac{\pi D^4}{64} \qquad (3)$$

The force required to bend a micro/ nanoscale pillar with a fixed base is given by the Euler-Bernoulli formula:

$$\frac{F}{\delta} = \frac{3\pi E D^4}{64L^3} \qquad (4)$$

Where, L is pillar height, D is pillar diameter, E is Young's modulus, and δ is deflection in pillar

Shear coefficient ($\boldsymbol{\alpha}$) for pillars with a circular cross-section, as a function of the Poisson's ratio (v) of the material is given by [7] :

$$\boldsymbol{\alpha} = \frac{6(1+v)^2}{7+12v+4v^2} \qquad (5)$$

The relationship between G and E is given by:

$$\boldsymbol{G} = \frac{E}{2(1+v)} \qquad (6)$$

The force required to bend a pillar by a distance δ is highly dependent on the relationship between the diameter *D* and the height *L*. High aspect ratio pillars seem to have high deflection distance. Thus, indicates that low force is needed to bend pillars with higher length compared to diameter.

Hence, the deflection for a single cylindrical pillar is:

$$\boldsymbol{\delta} = \boldsymbol{F}\frac{64L^3}{3\pi E D^4} + \boldsymbol{F}\frac{4\alpha L}{G\pi D^2} \qquad (7)$$

$$\boldsymbol{\delta} = \frac{8F}{\pi E}\left[\frac{8L^3}{3D^4} + \frac{(1+v)\alpha L}{D^2}\right] \qquad (8)$$

Apparent tissue Modulus ($E_{CELL\,TISSUE}$)for micro/nano pillar arrays

The objective in creating the appropriate polymeric substrate is to match the tissue's elastic moduli would sense of a particular cell with the apparent modulus created by the network of micro and nanoscale pillars. The approach involves equating the deflection ($\boldsymbol{\Delta x}$) caused by the cellular force (F_{CELL}) of a cell covering a FA area (A_{FA}) of a flat substrate to the previously derived deflection of an array of pillars ($\boldsymbol{\delta}$) that would lie within A_{FA}. This concept is shown in Figure 2.The main assumption of the model considers that the equivalent cellular force at a FA is equally divided among the number of pillars that would lie within the area of the FA, and hence each pillar would deflect at the same magnitude.

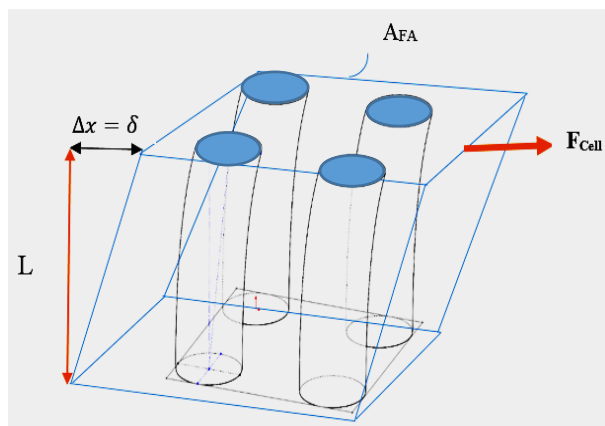

Figure 2: Injection Molded Array of Bending Micro/Nano Structures due to Force Exerted by Cell.

If a human stem cell is in contact with an extra cellular matrix (ECM) of a particular tissue, the cell will deform the tissue such that Δx is dependent on

the properties of the tissue. The shear force ($\boldsymbol{\tau}$)to shear strain ($\boldsymbol{\gamma}$) relationship is given by:

$$\boldsymbol{\tau} = \boldsymbol{G_{CELL\,TISSUE}\gamma} \qquad (9)$$

Where $\boldsymbol{G_{CELL\,TISSUE}}$ is the shear modulus of the targeted cell tissue, and for small angles of deflection($\boldsymbol{\alpha}$), and $\boldsymbol{\gamma} = \frac{\Delta x}{L}$.

Hence,

$$\frac{F_{CELL}}{A_{FA}} = G_{CELL\,TISSUE}\frac{\Delta x}{L} \qquad (10)$$

$$\Delta x = \frac{F_{CELL}\ L}{G_{CELL\,TISSUE}\ A_{FA}} \qquad (11)$$

Where,

$$G_{CELL\,TISSUE} = \frac{E_{CELL\,TISSUE}}{2(1+\nu_{CELL\,TISSUE})} \qquad (12)$$

For human embryonic cells, we assume that A_{FA} = 100 μm^2 , $\nu = 0.5$ [8] .

$$G_{CELL\,TISSUE} = \frac{E_{CELL\,TISSUE}}{3} \qquad (13)$$

The number of pillars per unit area ($\boldsymbol{\rho_{pillars}}$) can be written as a function of the pitch,

$$\rho_{pillars} = \frac{A_{FA}}{\chi^2} \qquad (14)$$

Hence, with the assumption that all pillars bear equal force, the force that is exerted at the tip of each pillar is given by,

$$F_{PILLAR} = \frac{F_{CELL}}{\rho_{pillars}} = \frac{F_{CELL}\,\chi^2}{A_{FA}} \qquad (15)$$

Since $\Delta x = \boldsymbol{\delta}$

$$\frac{F_{CELL}\ L}{G_{CELL\,TISSUE}\ A_{FA}} = \frac{8F_{PILLAR}}{\pi E}\left[\frac{8L^3}{3D^4} + \frac{(1+\nu)\alpha L}{D^2}\right] \qquad (16)$$

$$\frac{3F_{CELL}\ L}{E_{CELL\,TISSUE}} = \frac{8F_{CELL}\,\chi^2}{\pi E}\left[\frac{8L^3}{3D^4} + \frac{(1+\nu)\alpha L}{D^2}\right] \qquad (17)$$

$$E_{CELL\,TISSUE} = \frac{3\ \ \pi E}{8\chi^2}\left[\frac{8L^2}{3D^4} + \frac{(1+\nu)\alpha}{D^2}\right]^{-1} \qquad (18)$$

Where E is the elastic modulus and $\boldsymbol{\nu}$ is Poisson ratio of the substrate material. This final equation relates the manufactured substrate's intended elastic modulus to be mimicked as a function of the polymer surface's geometric (L, D, and$\boldsymbol{\chi^2}$) and material properties ($\boldsymbol{\alpha}$ and $\boldsymbol{\nu}$).

Results and Discussion

When creating the model a few different assumption were made to match human uterus elasic modulaus of 1.4 Mpa. Circular geomtries were chosen with the diamter of 5μm with different aspect ration and pitch to reach disiered appaernt cell tissue ($\boldsymbol{E_{CELL\,TISSUE}}$). Figure *3* shows that the presence of shear provides additional pillar deflection resulting in a lower apparent elastic modulus. This difference becomes fairly small when aspect ratio becomes greater than 2.

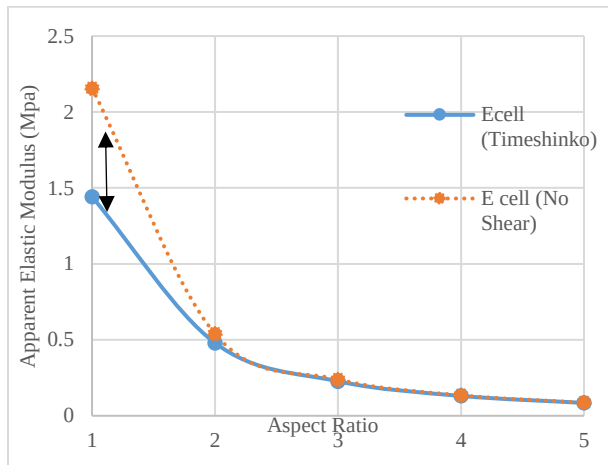

Figure 3: Effect of shear on apparent elastic modulus of 5um diameter, 8 um center to center spacing made of TPU with E=12.5 MPa.

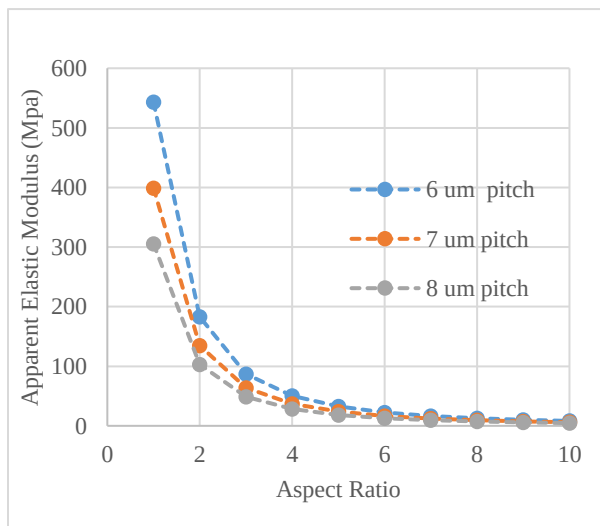

Figure 4: 5 um diameter Cylindrical Polystyrene pillars with 8um pitch.

Polystyrene substrates (which widely used in the petri- dishes industry), even with micro topography have shown that the apparent modulus is too high for the cells lying on the top of it to mimic a majority of biological tissues as seen in Figure 4. The demand for mimicking substrates with lower modulus of elasticity directed us to softer materials such thermoplastic polyurethane (TPU), Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), and other thermoplastic polymers with low elastic modulus as shown in Figure 5. Pillars spacing is an effective approach to reduce the amount of force that cells might exert as we see in Figure 6 but the amount of change reduces as the aspect ratio reaches the value of 5.

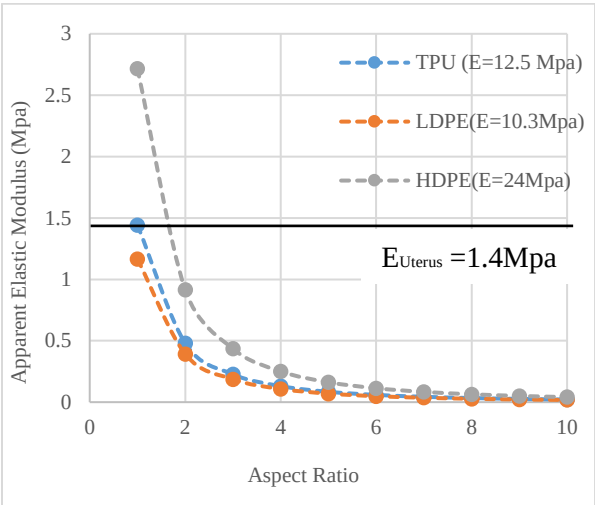

Figure 5: 5um diameter cylindrical pillars with 8 um pitch with different material.

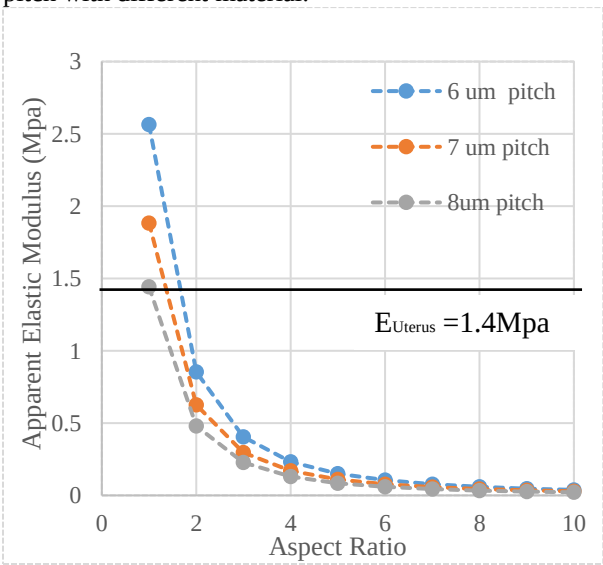

Figure 6: TPU 5um diameter Cylindrical pillars with different pitch.

Patterns dimensions are mainly affecting the apparent moduli on cells. As noted from Figure 7-Figure 8, the pillars diameter and height have smaller effect compared to spacing between pillars which could be the easiest way to manipulate the targeted apparent moduli. As the spacing between pillars increases a huge drop in $E_{CELL\ TISSUE}$ occurs due to inversely proportional relationship shown in Equation (18). High aspect ratio is a huge challenge when fabricating such substrates with micro/nano pillars. So, controlling spacing between pillars could provide an easier approach to fabricate substrates that match the modulus of the human body compared to high aspect ratio.

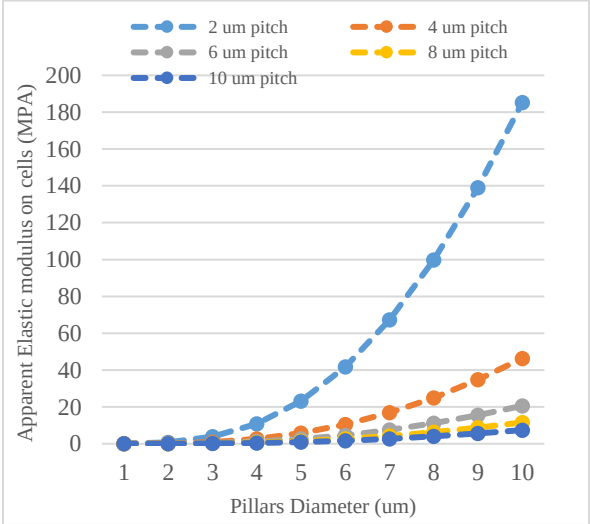

Figure 7: Pillars Diameter (D) sensitivity analysis to Apparent Elastic modulus on cells.

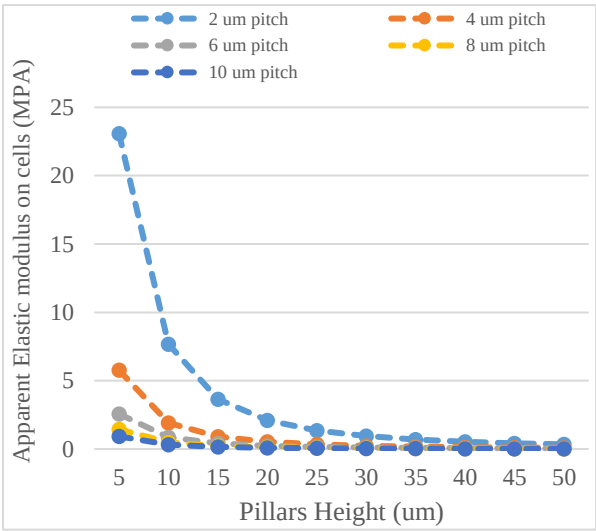

Figure 8: Pillars Height (L) sensitivity analysis to Apparent Elastic modulus on cells.

CONCLUSION

Analytical cell sensing model showed here will tolerate a proper understanding of pillar deflection and shear occur during cells culturing process based on Euler-Bernoulli and Timoshenko approximations. Such model will provide a better method to design and replicate pillar arrays prior to cell culturing procedure and significantly improve the ability to study cells developments. There are many variables that govern the apparent surface stiffness including shapes, aspect ratio, center to center spacing, and young modulus of the substrate. In this study, a novel of produce an appropriate elastic moduli that cell will sense in order to provide an equivalent modulus as target biological tissues. As noticed from derived expression that the presence of shear deformation offers additional pillar deflection resulting in a lower apparent modulus. We see that increasing the spacing between pillars (pitch) can be used to manipulate the targeted cell modulus for the replicated pattern. Material selection is also another variable of targeted modulus as we bare from this investigation polystyrene wouldn't be the proper choice when targeting lower apparent modulus.

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